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Activity of Penciclovir against Epstein-Barr Virus

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Penciclovir inhibited the productive replication cycle of Epstein-Barr virus (EBV) in assays measuring infectious virus production, viral antigen expression, and viral DNA synthesis. In the test measuring inhibition of EBV DNA synthesis, 50% effective concentrations of penciclovir and acyclovir were 2.3 ± 0.8 and 2.2 ± 0.6 μg/ml, respectively. The 50% cell growth inhibitory concentration of penciclovir was >100 μg/ml for both P_3HR-1 and Raji cells. Penciclovir is a selective inhibitor of EBV in cell culture.

Penciclovir [9-(4-hydroxy-3-hydroxymethylbut-1-yl)guanine (BRL 39123)] is a selective inhibitor of herpes simplex virus (HSV) and varicella-zoster virus in cell culture (6, 7). In addition, penciclovir has antiviral activity in several animal models (5), as does famciclovir (30), the oral prodrug of penciclovir. Famciclovir has recently been approved for the treatment of herpes zoster in immunocompetent patients, and its use is also being evaluated for patients with HSV infections. Penciclovir is phosphorylated selectively by the HSV and varicella-zoster virus thymidine kinases, and the resulting monophosphate is phosphorylated by cellular kinases to penciclovir-triphosphate, which inhibits viral DNA polymerase (3, 13, 33). Acyclovir inhibits Epstein-Barr virus (EBV) DNA replication (10, 11, 19) and was compared in this study with penciclovir, which has been reported to reduce the expression of the virus capsid antigen of EBV (6). This work confirms and extends the observation that penciclovir inhibits the productive replication cycle of EBV in cell culture.

Compounds and cell lines. Stock solutions of penciclovir and acyclovir for antiviral assays were prepared at 10 mg/ml in dimethyl sulfoxide and stored at −20°C. For cytotoxicity assays, compounds were dissolved at 1 mg/ml in growth medium alone and filter sterilized (0.2-μm-pore-size filters) prior to use.

Ramos (human B-lymphocyte origin; EBV negative) (18), Raji (human B-lymphocyte origin; latently infected with EBV) (26), P_3HR-1 (human B-lymphocyte origin; productively infected with an EBV deletion mutant) (16), and B95-8 cells (marmoset B-lymphocyte origin; productively infected with an EBV strain which immortalizes human B lymphocytes) (23) were cultured in growth medium (RPMI 1640 buffered with 25 mM HEPES [*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid], supplemented with 10% fetal calf serum, penicillin [50 IU/ml], and streptomycin [50 μg/ml]) at 37°C. The initial seeding rate was 2×10^5 cells per ml. Cell lines were provided by D. H. Crawford, London School of Hygiene and Tropical Medicine, London, United Kingdom.

Virus yield reduction assay. B95-8 cells were seeded onto 24-well plates at 1 ml per well and treated with penciclovir or acyclovir at 1 and 10 μg/ml for 7 days ($n = 4$). Cells were removed from pooled virus-containing supernatants by centrifugation at 100 × *g* for 20 min and filtration (0.45 μm-pore-size filters). These samples were stored at −70°C. Infectivity titers were determined by immortalization assays with peripheral blood mononuclear cells from volunteers who were seronegative for EBV, as assessed by the absence of antibodies to the viral capsid antigen (15). Mononuclear cells were separated on Ficoll-Paque gradients (Pharmacia Biotech, St Albans, United Kingdom), washed, and then exposed to 10-fold dilutions of test samples for 90 min at 37°C (0.1 ml of virus per 2×10^6 cells). Cells were washed again and plated out onto 96-well microtiter plates at 200 μl per well ($n = 5$). Cultures were refed at weekly intervals and scored for microscopic evidence of proliferation of immortalized lymphoblastoid cells between 4 and 5 weeks after infection. At 1 μg/ml, both compounds were inactive, but at 10 μg/ml, the virus yield relative to that of the untreated virus control was reduced by 94 and 86% by penciclovir and acyclovir, respectively (results not shown). From these data, the estimated 50% effective concentration (EC_{50}) was 3 to 4 μg/ml for each compound.

Antigen expression assay. The effects of penciclovir and acyclovir on EBV antigen expression were measured by an indirect enzyme-linked immunosorbent assay (ELISA). P_3HR-1 and Raji cells were grown on microtiter plates with penciclovir or acyclovir for 5 days ($n = 3$). Ramos cells were cultured in parallel in the absence of these compounds to serve as an EBV-negative control. Plates were centrifuged at 170 × *g* for 10 min, supernatants were removed, and cells were incubated for 1 h at 37°C in 50 μl of 1% Triton X-100 in phosphate-buffered saline per well. Solubilized antigen extracts were transferred to Immulon II plates (Dynatech Laboratories, Billingshurst, United Kingdom) and adsorbed overnight at 4°C. Viral antigens bound to plates were measured by an ELISA based on a method described elsewhere (27), with serum from a donor who was seropositive for EBV and with peroxidase-conjugated goat anti-human immunoglobulin G serum (Sigma Chemical Co., Ltd., Poole, United Kingdom). A_{450}s were read (Dynatech Mini Reader II). Extracts from Ramos control cultures were used to set the baseline absorbance, and test data were plotted as absorbance versus $\log_{10}$ compound concentration. The EC_{50} was calculated from the point at which the dose-response curve intersected the line representing 50% EBV antigen expression, which was calculated as the midpoint between the absorbance for extracts from untreated P_3HR-1 cells and that for extracts from untreated Raji cells. By using the latter value in the calculation, any contribution to the measured absorbances of test samples from the expression of the EBV nuclear antigen by latently infected cells was taken into account.

In a preliminary experiment, it was shown that the ELISA was specific for EBV because serum from an EBV-seropositive

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donor showed 11-fold greater reactivity against P_3HR-1 cell extracts than did serum from a seronegative donor, with both sera diluted 1:1,000. These two compounds had similar activities in P_3HR-1 cells and reduced antigen expression significantly at 3 μg/ml, compared with that of the untreated control ($P < 0.05$; t test). Presumably because only a small proportion of P_3HR-1 cells were productively infected at any particular time, the dose-response curves for these two compounds fell within a narrow range of absorbance readings. The EC_{50}s of penciclovir and acyclovir were between 1 and 3 μg/ml. Although a difference between penciclovir and acyclovir was observed at lower concentrations (1.0 to 0.1 μg/ml), with acyclovir-treated cultures appearing to express more EBV antigen than did control untreated cultures, this was not considered to be biologically significant. It was recently reported that acyclovir was more active than penciclovir against EBV in an ELISA measuring the late viral protein gp350, although no information was supplied on the cell culture system used or the treatment period (20). The mean 50% inhibitory concentrations were 5 μM (1.1 μg/ml) and 30 μM (7.6 μg/ml), respectively. These observations are contrary to our own data, which show that EBV is equally susceptible to both compounds by several different assays.

Infection of B lymphocytes by EBV usually results in latency. Transcription of the EBV genome is limited, cellular DNA polymerase replicates the virus genome, and the number of copies of the EBV genome per cell remains constant (17). As expected from earlier studies of acyclovir (10, 19), penciclovir did not affect established latent EBV infection, since the level of EBV antigen expression in Raji cells treated for 5 days with penciclovir at 10 μg/ml remained unchanged (results not shown).

EBV DNA inhibition assay. P_3HR-1 cells were treated with penciclovir or acyclovir, as described above for the viral antigen inhibition assay. Untreated Ramos cells were also included in this test. After 5 days, cell suspensions were transferred onto Biotrace filters (Gelman Sciences, Northampton, United Kingdom) by using a Hybridot manifold (Life Technologies Ltd.). Filters were air dried, DNA was denatured, and EBV DNA levels were quantitated by hybridization with a ^{32}P-labelled EBV-specific DNA probe as described previously (14, 34). The EBV probe consisted of the *Bam*G fragment of the EBV genome cloned into pBR322 (2), kindly provided by B. E. Griffin (Royal Postgraduate Medical School, Hammersmith, London, United Kingdom). In a preliminary experiment, it was shown that the DNA probe was present in excess during the hybridization reaction. To compare the relative activities of penciclovir and acyclovir, the EC_{50} for inhibition of EBV DNA synthesis, relative to that of the untreated virus control, was determined from graphs of DNA probe bound (expressed in counts per minute) plotted against $\log_{10}$ compound concentration. Treatment of EBV-infected cells with acyclovir eliminates the linear forms of EBV DNA associated with productive infection but does not affect the covalently closed circular DNA associated with latent infection (10, 11). However, the Ramos cell line, which is not infected with EBV, gave an acceptable background in the hybridization assay and was therefore used as a negative control to calculate the 50% endpoint.

The hybridization assay was specific for EBV DNA since the probe did not bind readily to DNA prepared from Ramos cells (EBV negative), whereas there was strong binding to DNA from untreated P_3HR-1 cells (Fig. 1). Penciclovir and acyclovir were almost equally active in this EBV DNA inhibition assay, with EC_{50}s of 1.5 and 1.6 μg/ml, respectively, following treatment of P_3HR-1 cells for 5 days (Fig. 1). This test was repeated for three subsequent experiments, giving an overall mean EC_{50}

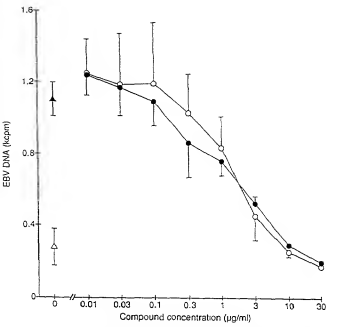

FIG. 1. Inhibition of EBV DNA synthesis in P_3HR-1 cells by penciclovir (●) and acyclovir (○). Cells were seeded at 2×10^5/ml and grown for 5 days with these test compounds. Parallel cultures of P_3HR-1 and Ramos cells were grown in the absence of compounds to serve as positive (▲) and negative controls (△), respectively. EBV DNA levels were determined by DNA hybridization with a radiolabelled DNA probe specific for EBV. Means of triplicate observations with standard deviations are shown.

(± standard deviation) of 2.3 (±0.8) μg/ml for penciclovir and of 2.2 (±0.6) μg/ml for acyclovir. Colby et al. (10) calculated an EC_{50} of 6 μM (1.4 μg/ml) for acyclovir against EBV DNA synthesis in P_3HR-1 cells, although a value of 0.3 μM (0.07 μg/ml) was reported by Lin et al. (19) after treatment of P_3HR-1 cells for 14 days with acyclovir. Both the duration of treatment employed in the latter instance and the baseline set for the DNA hybridization assay (19) would cause a reduction in the EC_{50}, relative to the figure reported by Colby et al. (10).

Cytotoxicity assays. Raji and P_3HR-1 cells were treated with the test compounds at 100 and 10 μg/ml ($n = 3$) for up to 5 days at 37°C. At daily intervals, cell suspensions were disaggregated by repetitive pipetting and cells were counted (Coulter Electronics, Harpenden, United Kingdom). Although P_3HR-1 cells were more sensitive to both penciclovir and acyclovir than Raji cells (Fig. 2), the 50% inhibitory concentration for either compound on day 5 was >100 μg/ml. There was some indication that penciclovir was more inhibitory for Raji cells than acyclovir was at 10 and 100 μg/ml, but the 50% inhibitory concentrations for both compounds were >100 μg/ml. In view of the wide therapeutic ratios for penciclovir and acyclovir, it is clear that both compounds are selective inhibitors of EBV.

The activity of penciclovir against productive EBV replication was compared with that of acyclovir in three different test systems, all of which are limited by the fact that only a minority of cells in the cultures are lytically infected. Nonetheless, penciclovir and acyclovir were shown to be potent inhibitors of infectious virus production, viral antigen expression, and viral DNA synthesis.

Mode of action studies are required to explain the activity of penciclovir against the productive replication cycle of EBV. With acyclovir, there is some uncertainty about the mode of

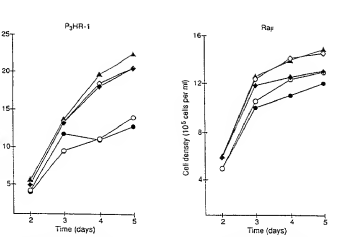

FIG. 2. Effects of penciclovir and acyclovir on replication of P_3HR-1 and Raji cells. Cells were seeded at 2×10^5/ml and treated with these test compounds. At the times indicated, cells from triplicate cultures were pooled and counted. The cell densities of cultures are plotted against time. ●, penciclovir, 100 μg/ml; ○, acyclovir, 100 μg/ml; ◆, penciclovir, 10 μg/ml; ◇, acyclovir, 10 μg/ml; ▲, control.

action. Although EBV encodes a thymidine kinase (21, 22), acyclovir is a very poor substrate for this enzyme (21, 31). Intracellular levels of acyclovir-triphosphate in EBV-infected cultures are low (9), but since only a small minority of cells within test cultures would have been productively infected, this is expected. Because acyclovir-triphosphate is a highly potent inhibitor of EBV DNA polymerase (8, 11), even if the extent of phosphorylation within infected cells is limited, sufficient acyclovir-triphosphate may be produced to inhibit the viral DNA polymerase. Penciclovir-triphosphate is between 10- and 20-fold more stable than acyclovir-triphosphate is within cells infected with HSV (13, 34); consequently, penciclovir causes more prolonged inhibition of HSV replication than does acyclovir (4). Whether this difference in intracellular stability also applies to cells which are infected with EBV is unknown. Lin et al. (19) reported that unlike acyclovir, ganciclovir [9-(1,3-dihydroxy-2-propoxymethyl)guanine] showed prolonged activity against EBV. Ganciclovir-triphosphate may have a longer half-life than that of acyclovir-triphosphate within EBV-infected cells, as has been shown for HSV (29).

Acyclovir has been used to treat patients with acute EBV-associated infectious mononucleosis. Intravenous acyclovir was shown to reduce oropharyngeal replication during the treatment period, although there was no clear evidence of symptomatic benefit (1, 24). By contrast, oral acyclovir (600 mg five times daily for 10 days) had only marginal effects on the suppression of EBV excretion in the oropharynx and no clinical benefit (32). However, the oral absorption of acyclovir is low (10 to 20%) and variable (12). Nonetheless, acyclovir given orally resulted in regression of EBV-associated oral hairy cell leukoplakia in a group of patients infected with human immunodeficiency virus, although recurrences occurred after cessation of treatment (28). An oral form of penciclovir, famciclovir, which gives high (77%) and consistent bioavailability of penciclovir has been developed (25). Clinical studies to evaluate the efficacy of famciclovir for the treatment of patients with either infectious mononucleosis or hairy cell leukoplakia are warranted by the selective inhibition of EBV by penciclovir demonstrated in cell culture.

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Antiherpesvirus Activity of 9-(4-Hydroxy-3-Hydroxymethylbut-1-yl)Guanine (BRL 39123) in Cell Culture

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The activity of 9-(4-hydroxy-3-hydroxymethylbut-1-yl)guanine (BRL 39123) against several herpesviruses was compared with that of acyclovir (ACV). In plaque reduction tests with clinical isolates of herpes simplex virus type 1 (HSV-1), herpes simplex virus type 2 (HSV-2), and varicella-zoster virus, mean 50% inhibitory concentrations (IC_{50}s) (n = number tested) for BRL 39123 were 0.4 (n = 17), 1.5 (n = 13), and 3.1 (n = 5) μg/ml, respectively. Corresponding IC_{50}s for ACV were 0.2, 0.6, and 3.8 μg/ml. Cytomegalovirus was relatively resistant to BRL 39123 (IC_{50}, 51 μg/ml), but equid herpesvirus 1, bovid herpesvirus 2, and felid herpesvirus 1 were susceptible (IC_{50}s, 1.6, 1.2, and 0.9 μg/ml, respectively). BRL 39123 was inactive against an HSV-1 strain which does not express thymidine kinase activity, but a DNA polymerase mutant selected for resistance to ACV was sensitive to BRL 39123 (IC_{50}, 1.5 μg/ml). In contrast to the results from plaque reduction tests, BRL 39123 was more active than ACV against HSV-1 and of equal activity against HSV-2 in virus yield reduction assays in MRC-5 cells. After treatment of HSV-infected cultures for short periods, BRL 39123 was considerably more effective than ACV at reducing virus replication, and furthermore, after removal of extracellular BRL 39123, virus replication remained depressed for long periods, whereas such persistent activity was not observed with ACV. Neither compound significantly affected MRC-5 cell replication at 100 μg/ml, but at 300 μg/ml BRL 39123 was more inhibitory than ACV.

Since the discovery of acyclovir (ACV) and its introduction as an effective treatment for herpes simplex virus types 1 and 2 (HSV-1 and HSV-2) and varicella-zoster virus (VZV) infections in humans, several laboratories have been searching for acyclic purine nucleosides with improved properties. ACV is a highly selective antiviral agent which is activated by the virus-induced thymidine kinase and inhibits susceptible viruses at concentrations which do not affect cell replication (9). Several other guanine derivatives have been described and evaluated as inhibitors of herpesviruses in cell culture and in animal infections (1, 10, 13, 14, 22–24). Many publications show that 9-(1,3-dihydroxy-2-propoxymethyl)guanine (DHPG) is beneficial in the treatment of severe cytomegalovirus (CMV) infections in humans, but because of adverse toxicity its use in less serious infections may be limited (20).

Pandit et al. (18) first claimed to have synthesized 9-(4-hydroxy-3-hydroxymethylbut-1-yl)guanine, but the compound was not fully characterized and no biological properties were given. The synthesis and isolation of pure compound have since been reported by Harnden and Jarvest (12), who found that the experimental conditions originally described provided a mixture of products.

In the present paper, the activity of 9-(4-hydroxy-3-hydroxymethylbut-1-yl)guanine (BRL 39123; Fig. 1) is compared with that of ACV in plaque reduction assays against veterinary and human herpesvirus isolates, including recent clinical isolates of HSV and VZV and also two ACV-resistant strains of HSV-1. BRL 39123 and ACV have also been evaluated for their inhibitory effects on virus replication in virus yield reduction assays. The data in this report extend the observations made independently by Larsson et al. (15), MacCoss et al. (16), and Tippie et al. (24) on the biological activity of 9-(4-hydroxy-3-hydroxymethylbut-1-yl)guanine, the compound we have called BRL 39123.

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MATERIALS AND METHODS

Compounds. The guanine derivatives BRL 39123 (12) and ACV (19) were prepared within the chemical laboratories of Beecham Pharmaceuticals. Compounds were dissolved at 10 mg/ml in dimethyl sulfoxide and stored at −20°C prior to dilution in cell culture medium.

Cell cultures. EBTr, RK-13, and Vero cells (Flow Laboratories Ltd., Irvine, Scotland) were grown in Eagle minimal essential medium (MEM) with 10% newborn calf serum (NCS). BHK-21 cells (Flow) were grown in Glasgow MEM containing 10% NCS and 10% tryptose phosphate broth. MRC-5 cells (National Institute for Biological Standards and Control, Holly Hill, London, United Kingdom) and MDBK cells (Central Veterinary Laboratories, Weybridge, United Kingdom) were grown in Eagle MEM with 10% fetal calf serum (FCS). FEA cells (feline embryonic type A cells; provided by D. A. Harbour, University of Bristol) were grown in Glasgow MEM containing 10% tryptose phosphate broth and 5% FCS. All media and sera were purchased from Gibco Ltd., Paisley, Scotland. BHK-21, MRC-5, and Vero cells, which were used extensively in this work, were cultured in the absence of antibiotics and were free from mycoplasma contamination.

Viruses. Stocks of HSV-1 and HSV-2 strains and also bovid herpesvirus type 2 (BHV-2; bovine mammillitis/strain New York 1; were prepared in BHK-21 cells. BHV-1, strain Oxford 1964 (infectious rhinotracheitis), equid herpesvirus type 1 (EHV-1), strain Quai Hais/equine rhinopneumonitis), and felid herpesvirus type 1 (FHV-1), strain B927 (feline rhinotracheitis), were grown in MDBK, RK-13, and FEA cells, respectively. Stocks of CMV and VZV were prepared in MRC-5 cells. HSV-1 strain CR101 and its ACV-resistant variants were supplied by H. J. Field (University of Cambridge, Cambridge, United Kingdom) (11).

Plaque reduction assays. Confluent cell monolayers in 24-well multidishes (well diameter, 1.5 cm; Gibco, Paisley,

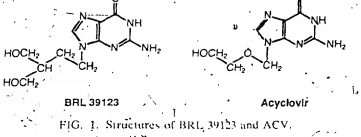

FIG. 1. Structures of BRL 39123 and ACV.

Scotland) were infected with about 50 PFU of virus in 100 μl of phosphate-buffered saline (PBS). After adsorption at room temperature for 1 h, residual inoculum was replaced with 0.5 ml of medium containing 5% NCS and 0.9% agarose (Indubiose A37; Uniscience Ltd., London, United Kingdom). Heat-inactivated FCS was used instead of NCS in plaque assays with VZV and CMV. Once the agarose had set, dilutions of the test compounds, which had been prepared in medium containing 5% NCS or FCS as appropriate, were added, each well receiving 0.5 ml of liquid overlay. The final concentrations of drugs achieved were 100, 30, 10, 3, 1, 0.3, 0.1, and 0.03 μg/ml. Infected cultures were incubated at 37°C in a humidified atmosphere of 5% CO_2–95% air until plaques were clearly visible. In CMV plaque reduction assays, which were incubated for about 9 days, each well contained 1 ml of agarose and 1 ml of liquid overlay. Cell monolayers were fixed in formol-saline and stained with carbol fuchsin, and plaques were counted with the aid of a stereomicroscope. By reference to the number of plaques observed in virus control monolayers (untreated cultures), the concentration of test compound which inhibited plaque numbers by 50% (IC_{50}) was calculated.

Virus yield reduction assays. Monolayers of MRC-5 cells prepared in microliter plates (Nunc) were infected with HSV-1 or HSV-2 diluted in PBS, 50 μl per well. After adsorption for 1 h at room temperature, residual inoculum was removed and cell monolayers were washed twice with PBS. Test compounds which had been diluted in assay medium (Eagle MEM plus 5% NCS) were added to give 0.2 ml per well. Plates were incubated at 37°C, and at various times after infection supernatants were collected, clarified by centrifugation (Eppendorf microcentrifuge; 2 min at 12,000 rpm), and stored at −70°C. In experiments in which infected cell cultures were treated with BRL 39123 and ACV for various times and subsequently reincubated in drug-free medium, cultures were washed twice with PBS before fresh medium was added. All experiments were performed in triplicate. Supernatants from replicate cultures were titrated individually on Vero cells, and the geometric mean infectivity titer was calculated.

In one series of experiments, several strains of HSV-1 and HSV-2 were tested for susceptibility to BRL 39123 and ACV. The compounds were added 1 h after infection, and the yield of virus 24 h after infection was measured. By reference to the amount of virus produced by virus control monolayers (untreated cultures), the concentration of compound required to inhibit the virus control yield by 99% (IC_{99}) was calculated.

Cytotoxicity assay. Effects on cell proliferation were studied by the addition of compounds in the absence of dimethyl sulfoxide to actively replicating MRC-5 cells. Cell numbers were measured after incubation for 72 h in a Coulter counter (Coulter Electronics, Harpenden, United Kingdom).

Statistical analysis. The analysis of variance was used to compare IC_{50}s for clinical isolates of HSV-1, HSV-2, and VZV and also to compare IC_{99}s derived from virus yield reduction assays. Differences between BRL 39123 and ACV in cytotoxicity assays were evaluated by Student's t test. A value of $P < 0.05$ was considered statistically significant.

TABLE 1. Activity of BRL 39123 and ACV against different herpesviruses measured by the plaque reduction assay

Virus (strain)	Cells	IC_{50} (μg/ml)	
		BRL 39123	ACV
HSV-1 (HFEM)	MRC-5	0.5	0.3
HSV-2 (MS)	MRC-5	0.8	0.4
VZV (Ellen)	MRC-5	2.4	3.3
CMV (AD-169)	MRC-5	52	25
BHV-1 (Oxford 1964)	EBTr	100	>100
BHV-2 (New York 1)	BHK-21	1.2	25
EHV-1 (Quai Ham)	RK-13	1.6	2.6
FHV-1 (B927)	FEA	0.9	11

RESULTS

Spectrum of activity. Several laboratory strains of herpesviruses were tested for susceptibility to BRL 39123 and ACV by the plaque reduction assay (Table 1). BRL 39123 was up to twofold less active than ACV against HSV-1 and HSV-2 but slightly more active than ACV against VZV. Both compounds showed minimal activity against CMV. Epstein-Barr virus (EBV) was susceptible to BRL 39123 and ACV in a test which measured the inhibition of virus capsid antigen expression, activity being observed at 10 μg/ml (data not shown). Greater differences between the two compounds were observed among veterinary herpesviruses (Table 1). BRL 39123 was active at about 1 μg/ml against BHV-2 and EHV-1, while the IC_{50} of ACV for both viruses exceeded 10 μg/ml. BRL 39123 was slightly more active than ACV against EHV-1, and both compounds were inactive against BHV-1.

Clinical isolates of HSV-1, HSV-2, and VZV were screened for susceptibility to BRL 39123 and ACV by the plaque reduction assay (Table 2). These viruses have undergone limited passage in vitro since isolation. By this method, BRL 39123 was significantly less active than ACV against HSV-1 ($P = 0.021$) and HSV-2 ($P < 0.01$) and was of equal activity against VZV. HSV-1 was significantly more susceptible to BRL 39123 than HSV-2 ($P < 0.01$), and HSV-2 was more susceptible than VZV ($P < 0.01$).

While both BRL 39123 and ACV showed limited activity against an HSV-1 strain which did not synthesize viral thymidine kinase, C1(101)TK⁻, BRL 39123 was active

TABLE 2. Activity of BRL 39123 and ACV against clinical isolates of HSV-1, HSV-2, and VZV in MRC-5 cells measured by the plaque reduction assay

Virus	No. of isolates tested[a]	Mean IC_{50} (μg/ml) ± SD	
		BRL 39123	ACV
HSV-1	17	0.4 ± 0.1	0.2 ± 0.2
HSV-2	13	1.5 ± 0.4	0.6 ± 0.2
VZV	5	3.1 ± 0.8	3.8 ± 0.7

[a] Clinical isolates of HSV-1 and HSV-2 were kindly provided by H. Cummin (Epsom Public Health Laboratory, West Park Hospital, Epsom, Surrey, United Kingdom) and M. M. Ogilvie (Southampton General Hospital, Southampton, United Kingdom). VZV strains were supplied by J. V. Membrane (Epsom Public Health Laboratory, West Park Hospital).

TABLE 3. Activity of BRL39123 and ACV against ACV-resistant mutants of HSV-1 Cl(101)

Virus strain	Phenotype	IC_{90} (μg/ml)[a]	
		BRL 39123	ACV
Cl(101)	Wild type	1.1	0.4
Cl(101)TK⁻	TK⁻	87	58
Cl(101)P_2C_5	TK⁺, DNA polymerase mutant (ACV[r])	1.5	21

[a] Plaque reduction assay in Vero cells.

against a DNA polymerase mutant, Cl(101)P_2C_5, which was resistant to ACV (Table 3).

Effects on HSV replication. BRL 39123 and ACV inhibited the replication of HSV-1 and HSV-2 in the virus yield reduction assay in MRC-5 cells; the extent of inhibition was related to the concentration of compound (Fig. 2). The IC_{90}s (concentrations required to reduce the yield of infectious virus obtained 24 h after infection by 99% relative to control cultures) derived from the data in Fig. 2 were 0.4 and 1.0 μg/ml for BRL 39123 and ACV, respectively, against HSV-1. The corresponding figures for HSV-2 were 0.7 μg/ml for BRL 39123 and 0.9 μg/ml for ACV. Several other clinical isolates have been tested in addition to the laboratory strains HSV-1 (HFEM) and HSV-2 (MS) (data not shown). The IC_{90}s were in good agreement with those quoted above. In virus yield reduction assays, BRL 39123 was thus more potent than ACV against HSV-1 strains ($P = 0.05$) and of equal activity against HSV-2 strains ($P = 0.77$).

In subsequent experiments the effect of varying the time of contact between the test compounds and cell monolayers infected with HSV-1 and HSV-2 was investigated. MRC-5 cells infected with HSV-2 (MS) were exposed to BRL 39123 and ACV at 30 μg/ml for various lengths of time beginning 2 h after infection, and cell-free virus titers were measured 25 h after infection (Table 4). Brief treatment with BRL 39123, for example between 2 and 6 h after infection, resulted in a considerably lower virus yield than equivalent treatment with ACV. The continuous presence of ACV was required to achieve maximum reduction in virus yield, whereas this was attained after 5 h of incubation with BRL 39123. Similar results were obtained with HSV-1 in MRC-5 cells.

TABLE 4. Effect of duration of treatment on the inhibition of HSV-2 (MS) replication in MRC-5 cells

Treatment period[a] (h postinfection)	Virus yield at 25 h[b] (PFU/0.1 ml)	
	BRL 39123	ACV
None	4.5×10^3	4.5×10^3
2–4	2.2×10^3	2.8×10^3
2–5	8.2×10^2	1.8×10^3
2–6	2.0×10^0	6.3×10^2
2–7	$<1.0 \times 10^0$	4.5×10^2
2–9	$<1.0 \times 10^0$	1.3×10^2
2–11	$<1.0 \times 10^0$	3.0×10^1
2–13	$<1.0 \times 10^0$	1.0×10^1
2–25	$<1.0 \times 10^0$	1.3×10^0

[a] Nucleoside analogs at 30 μg/ml were added 2 h after infection and replaced at various times with fresh assay medium.
[b] The MOI was 1.8 PFU per cell.

MRC-5 cells infected with HSV-2 were treated with BRL 39123 and ACV for 18 h after infection, and thereafter incubation was continued in the absence of the compounds. The amount of virus produced by these cultures was measured for up to 6 days (Fig. 3). After the removal of BRL 39123 at 3 μg/ml, the infectivity titers of supernatants from these cultures declined. In contrast, the amount of virus produced by infected cells after treatment for 18 h with ACV at 30 μg/ml rapidly increased to give levels similar to those released by untreated cells. Persistent antiviral activity in MRC-5 cells was observed with concentrations of BRL 39123 as low as 1 μg/ml. Furthermore, persistent antiviral activity of BRL 39123 was also observed in Vero cells infected with HSV-1 and HSV-2; although the effect was not as great as that described for MRC-5 cells (data not shown).

Cytotoxicity. No morphological abnormalities were observed in uninfected, established cell monolayers treated with either BRL 39123 or ACV at 100 μg/ml during the plaque reduction assays. However, to determine whether cell replication was adversely affected by these guanine derivatives, subconfluent monolayers of MRC-5 cells were grown in 24-well plates for 72 h with various concentrations of either BRL 39123 or ACV. At 100 μg/ml, neither compound significantly affected cell proliferation. At 300 μg/ml,

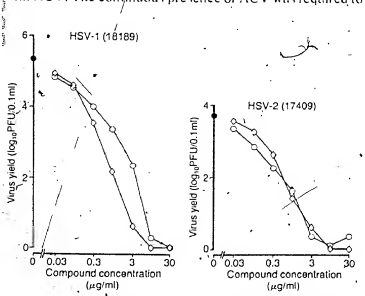

FIG. 2. Inhibition of replication of clinical isolates of HSV-1 and HSV-2 by the virus yield reduction assay in MRC-5 cells. Symbols: ◇, BRL 39123; ○, ACV; ●, virus control yield. The MOIs were 0.9 PFU per cell for HSV-1 and 0.4 PFU per cell for HSV-2. Nucleoside analogs were added 1 h after virus infection, and culture supernatants were harvested 24 h after infection.

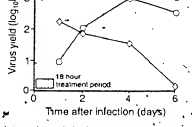

FIG. 3. Duration of antiviral activity after 18-h treatment of MRC-5 cells infected with HSV-2 (MS). Symbols: ◇, BRL 39123; ○, ACV; ●, untreated virus control. The concentrations of BRL 39123 and ACV were 3 and 30 μg/ml, respectively. The MOI was 0.7 PFU per cell. Compounds were added 1 h after infection and replaced 18 h later with fresh assay medium. Infectivity titers of culture supernatants collected at the times indicated were measured.

both compounds were significantly different from the control ($P < 0.05$), and BRL 39123 was more inhibitory than ACV ($P < 0.05$). The mean numbers of population doublings in cultures treated at 300 μg/ml were 2.3 for BRL 39123 and 3.0 for ACV, compared with 3.2 for control cultures.

DISCUSSION

BRL 39123 is a selective inhibitor of herpesvirus replication, as demonstrated by the wide separation between the concentration which affected virus replication and that which affected cell proliferation. Our results show that BRL 39123 was active against herpesviruses expressing thymidine kinase, which implies that BRL 39123, like ACV, is converted by this enzyme to the monophosphate. Information on the biochemical mode of action of BRL 39123 is currently being obtained. Larsson et al. (15) recently reported that this compound is a substrate with high affinity for the viral thymidine kinase and a potent inhibitor of viral DNA synthesis.

We used two methods to obtain information on the relative potency and properties of BRL 39123 and ACV, the plaque reduction assay and the virus yield reduction assay (6). The results of plaque reduction assays indicate that BRL 39123 and ACV have a similar spectrum of activity but that there are differences in relative potency. Our independent assessment of the properties of BRL 39123 is in accord with that of others (15, 16, 24) and shows that the compound is a potent inhibitor of HSV-1 and HSV-2. In addition, we report that BRL 39123 is active against VZV. In plaque reduction assays against a range of clinical isolates, BRL 39123 was less active than ACV against HSV-1 and HSV-2, of equal activity against VZV, and active against three veterinary viruses, BHV-2, EHV-1, and FHV-1. As reported previously (15, 16, 24), our results indicate that in plaque reduction assays BRL 39123 is less active against HSV-2 than HSV-1. MacCoss et al. (16), however, reported 50% effective doses of 25 to 50 μM for HSV-1 and 200 μM for HSV-2, which are much higher than our values and those of others (15, 24). The reason for this discrepancy is unclear.

ACV inhibits EBV DNA replication in P3HR-1 cells and reduces the percentage of cells expressing capsid antigen (5). Our observations suggest that the expression of EBV capsid antigen was equally susceptible to inhibition by BRL 39123 and ACV. In accord with the results of Tippie et al. (24), we also found that BRL 39123 had limited activity against CMV. In contrast, DHPG, which is structurally related to BRL 39123, is very active against CMV (4, 17). The mechanism of activity of DHPG against CMV is poorly understood, since CMV is not known to express thymidine kinase, but it has been suggested that a cell enzyme could perform the initial phosphorylation of DHPG (3). Whatever the mechanism of action of DHPG against CMV, it does not appear to be the same as that of BRL 39123.

There was no evidence of intrinsic resistance to BRL 39123 among the clinical strains of HSV or VZV tested, which were from several sources. Similar findings have been reported for ACV (2, 8). However, it was noted that BRL 39123 inhibited an ACV-resistant strain of HSV-1, C(101)P$_2$C$_4$, which carries a mutation in the DNA polymerase gene. This suggests that either the two compounds interact with the DNA polymerase at different sites or that the affinity of the enzyme for BRL 39123 differs from that for ACV.

In virus yield reduction assays under conditions of relatively high multiplicity of infection (MOI) (about 1 PFU per cell), it was observed that BRL 39123 was more active than ACV against HSV-1 strains and equally active against HSV-2 strains. Results from yield reduction assays thus change the assessment of the relative potency of these two compounds compared with data obtained by plaque reduction assays. Two important differences between the tests concern the MOI and the nature of the endpoint; a low virus challenge was used in the plaque reduction assay, whereas cells were infected at a high MOI in the virus yield reduction assay, and in the latter assay infectious virus production was measured rather than plaque formation. Antiviral agents are often ranked according to their performance in plaque reduction tests (2, 7), but it may be of greater relevance to chemotherapy to test their ability to inhibit the production of infectious virus, as in the virus yield reduction assay.

Two further interesting phenomena emerged during our investigations of the activity of BRL 39123 against HSV. First, maximum inhibition of virus replication was achieved following comparatively short treatment with BRL 39123, whereas the presence of ACV was required throughout the incubation period to obtain the same inhibition. Second, once inhibition of virus replication was achieved by treatment with BRL 39123, virus replication remained depressed for long periods after extracellular compound had been removed from the cultures. In contrast, virus replication rapidly resumed after removal of extracellular ACV. These two findings may reflect the efficient trapping of the phosphorylated form of BRL 39123 in the infected cell. In addition, BRL 39123 may be phosphorylated more rapidly than ACV, and this might account for the faster generation of the antiviral state in cultures treated with BRL 39123. For example, Field et al. (10) found that in crude extracts of HeLa cells infected with HSV-1, the rate of phosphorylation of DHPG was much faster than that of ACV.

Our results concerning the persistent activity of BRL 39123 are similar to those of Cheng et al. (4), who found that treatment of HSV-1-infected cultures with DHPG up to 8 h after infection was as effective as having the compound present throughout the incubation period. It was also shown (21) that DHPG triphosphate in HSV-2-infected cells had greater stability than ACV triphosphate. A similar trapping mechanism has been proposed for buciclovir [(*R*)-9-(3,4-dihydroxybutyl)guanine] (15). If also true for BRL 39123, this could be clinically advantageous when a drug is rapidly removed from the circulation or site of administration; effective treatment with BRL 39123 may not be dependent on maintenance of antiviral drug concentrations in the bloodstream.

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NOTES

Antiviral Drug Susceptibility of Human Herpesvirus 8

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We studied the susceptibility of human herpesvirus 8 (HHV-8) to a number of antiherpesvirus agents. The acyclic nucleoside phosphonate (ANP) analogs cidofovir and HPMPA [(*S*)-1-(3-hydroxy-2-phosphonylmethoxypropyl)adenine] effected potent inhibition of HHV-8 DNA synthesis, with 50% effective concentrations (EC_{50}) of 6.3 and 0.6 μM, respectively. Adefovir, an ANP with both antiretrovirus and antiherpesvirus activity, blocked HHV-8 DNA replication at a fourfold-lower concentration than did foscarnet (EC_{50} of 39 and 177 μM, respectively). The most potent inhibitory effect was obtained with the N-7-substituted nucleoside analog S2242 (EC_{50}, 0.11 μM). The nucleoside analogs acyclovir, penciclovir, H2G {(*R*)-9-[4-hydroxy-2-(hydroxymethyl) butyl]guanine}, and brivudine had weak to moderate effects (EC_{50} of ≥75, 43, 42, and 24 μM, respectively, and EC_{90} of ≥75 μM), whereas ganciclovir elicited pronounced anti-HHV-8 activity (EC_{50}, 8.9 μM).

Recently a novel virus, Kaposi's sarcoma (KS)-associated herpesvirus, or human herpesvirus 8 (HHV-8), was discovered in an AIDS-KS lesion (3). HHV-8 is a gamma herpesvirus related to herpesvirus saimiri. HHV-8 sequences are detected in nearly all KS lesions (human immunodeficiency virus [HIV] related and unrelated) examined so far. Also, serological studies showed evidence that HHV-8 is specifically associated with KS (6). In addition, the virus is associated with a rare B-cell primary effusion (body cavity-based) lymphoma and some forms of Castleman's disease (2, 12).

HHV-8 replicates in mononuclear cells (5), and lytic infection in the KS lesion appears to occur only in a restricted number of cells of the KS lesion (21). Therefore, inhibition of the lytic cycle of HHV-8 may be expected to have little impact on the evolution of established KS. However, after the initial infection with HHV-8 the virus must replicate, at least in the immunodeficient host, thus amplifying the viral load in the body. It is thus conceivable that if (i) one can block the expansion of the HHV-8 population (this may be shortly after infection or at the time that the immunosuppression is sufficiently profound) and (ii) HHV-8 is indeed the etiological factor in KS and primary effusion lymphoma, then the load of HHV-8 may be too low to cause HHV-8-related malignancies or the risk of developing these malignancies may at least be reduced. From this perspective it is thus important to detect HHV-8 positivity as soon as possible following the initial infection and to start anti-HHV-8 therapy promptly with an effective, nontoxic compound. Alternatively, as suggested by Zhong et al. (21), if latent viral infection drives the proliferation of spindle cells in KS and if this stimulus is not sustained or limited by apoptosis, growth of the lesion would depend on the recruitment of newly infected cells.

Morfeldt and Torssander (10) observed that of five patients with HIV-associated KS, three went into long-term remission following foscarnet treatment. However, factors other than foscarnet may have influenced regression of KS. No follow-up study has been published. In a recent study it was found that HHV-8 DNA was not cleared from the peripheral blood mononuclear cells in five patients under foscarnet and/or ganciclovir (GCV) treatment (8). From a retrospective study of a large series of AIDS patients, it was concluded that the risk for KS was slightly increased with acyclovir (ACV) use, minimally affected with GCV use, and significantly decreased ($P < 0.001$) following foscarnet treatment (9). Some decrease in the risk of KS with GCV, but not ACV, was reported by Glesby et al. (7). It thus seems mandatory to collect information on the susceptibility of HHV-8 to existing antiviral drugs. This study not only may have a clinical impact on the therapy of HHV-8-associated lesions but also may provide deeper insight into the mode of replication of this virus.

We here examined the effects of different antiherpes molecules on the replication of HHV-8 in the 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-inducible HHV-8-containing BCBL-1 cell line (18). Exponentially growing BCBL-1 cells (NIH AIDS Research & Reference Reagent Program) were seeded at a density of 300,000 cells/ml in the absence or presence of 30 ng of the tumor promoter TPA (Sigma) per ml. Cultures were incubated with the different antiviral drugs for 7 days (5 ml of fresh medium containing TPA and antivirals was added at day 4), at which time total cellular DNA was extracted. The origin of the compounds is as described previously (1). Ten micrograms of denaturated total cellular DNA of drug-treated BCBL-1 and control cells were blotted onto a nylon membrane (Hybond-N, Amersham) and UV-cross-linked, after which prehybridization was carried out for 1 h at 42°C. The probe was labeled with digoxigenin-dUTP in a PCR; the 5′-to-3′ primer sequence was AGC CGA AAG GAT TCC ACC ATT and TCC GTG TTG TCT ACG TCC AGA, delimiting a 213-bp sequence within the viral capsid antigen region. The probe was gel purified, and hybridization was carried out for 18 h at 42°C with 30 ng of the digoxigenin-11-dUTP labeled probe per ml. The membrane was washed at high stringency (2× SSC [1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate]–0.1% sodium dodecyl sulfate) for 10 min at room temperature followed by two washes of 15 min each in 0.1× SSC–0.1% sodium dodecyl sulfate at 65°C. After incuba-

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TABLE 1. Inhibitory effects of selected compounds on HHV-8 replication[a]

Compound	EC (μM)[b]		CC_{50} (μM)[c]	SI[d]
	50%	90%		
ACV	≥75	<110	≥690	≤9.2
GCV	8.9 ± 3.5	23 ± 27	354 ± 171	39
PCV	43 ± 7.4	119 ± 24	132 ± 54	3.0
BVDU	24 ± 12	≥75	342 ± 105	≥13
S2242	0.11 ± 0.01	0.6 ± 0.5	24 ± 19	244
HPMPC	6.3 ± 1.8	18 ± 9	340 ± 111	54
HPMPA	0.6 ± 0.9	2.6 ± 1.6	191 ± 122	290
PMEA	39 ± 30	90 ± 0.0	82 ± 12	2.1
PFA[e]	177 ± 57	≥449	≥702	≥3.9
H2G	42 ± 31	≥97	471 ± 33	11

[a] Data are mean values for 3 or 4 independent experiments ± standard deviations

[b] EC_{50} and EC_{90}, concentrations required to reduce HHV-8 DNA synthesis in TPA-stimulated BCBL-1 cells by 50% and 90%, respectively.

[c] CC_{50}, 50% cytotoxic concentrations (concentrations required to reduce the growth of uninduced BCBL-1 cells by 50% [as evaluated over a 4-day period]).

[d] SI, selectivity index (ratio of CC_{50} to EC_{50}).

[e] PFA, foscarnet.

tion in blocking buffer, the filter was incubated with an anti-digoxigenin antibody and conjugated with alkaline phosphatase (anti-digoxigenin-AP and Fab fragments; Boehringer Mannheim), and detection of chemiluminescence was performed by standard methods. Because the antivirals (see above) had little or no effect on the signal in noninduced cells (only 1 to 3% of the BCBL-1 cells spontaneously enter the lytic cycle [10]), the background signal from the uninduced cultures was subtracted from that of the TPA-induced cultures and the 50% and 90% effective concentrations (EC_{50} and EC_{90}, respectively) were calculated by extrapolation from graphic plots.

The HHV-8 DNA content was increased on average 13.3 ± 2.5-fold (mean ± standard deviation) in the TPA-treated cultures compared to uninduced control cultures. As can be derived from Table 1, cidofovir {HPMPC [(*S*)-1-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine]} and HPMPA [(*S*)-9-(3-hydroxy-2-phosphonylmethoxypropyl)adenine] emerged as potent inhibitors of HHV-8 DNA replication, with EC_{50} of 6.3 ± 1.8 and 0.6 ± 0.9 μM, respectively, and reduced the growth of uninduced BCBL-1 cells by 50% only at concentrations of 340 and 190 μM, respectively, resulting in selectivity indices of 54 and 290. Ninety percent inhibition of HHV-8 DNA synthesis was achieved at 18 μM (5 μg/ml) cidofovir and 2.6 μM HPMPA. Figure 1 depicts the dose-dependent inhibition of HHV-8 DNA synthesis by cidofovir as well as the effect of the compound on the growth of uninduced cells. The HPMPA congener adefovir {PMEA [9-(2-phosphonylmethoxyethyl)adenine]}, a compound with both broad-spectrum antiretrovirus and antiherpesvirus activity, also caused marked inhibition of HHV-8 DNA synthesis (EC_{50}, 39 ± 30 μM). In fact, adefovir proved 4.4-fold-more potent as an anti-HHV-8 agent than foscarnet (EC_{50}, 177 ± 57 μM). Adefovir caused 90% inhibition of HHV-8 DNA synthesis at a concentration of 90 μM, whereas foscarnet did so only at concentrations of ≥449 μM. ACV, penciclovir (PCV), brivudine (BVDU), and H_2G {(*R*)-9-[4-hydroxy-2-(hydroxymethyl)butyl]guanine}, compounds that depend for their activation on a virus-encoded thymidine kinase (TK), had weak to moderate inhibitory effects on the lytic replication of HHV-8 (EC_{50} of ≥75, 43, 24, and 42 μM, respectively, and EC_{90} of ≥75 μM). Yet GCV conferred antiviral protection, with an EC_{50} of 8.9 μM (EC_{90}, 23 μM). The novel nucleoside analog S2242 [(1,3-dihydroxy-2-propoxymethyl)purine], which can be viewed as a 6-deoxy form of GCV with the acyclic side chain substituted at the N-7 rather than the N-9 position, emerged as the most potent anti-HHV-8 compound of this series, with an EC_{50} of 0.11 ± 0.01 μM and an EC_{90} of 0.6 ± 0.5 μM. When cidofovir and GCV (≈100 μM) were added to the TPA-induced cell cultures for only a 72-h period, followed by extensive washing of the cultures, cidofovir still caused complete inhibition of HHV-8 DNA synthesis when evaluated 7 days after TPA induction. By contrast, under this experimental condition GCV lost all protective effect (EC_{90}, >100 μM) (data not shown). Very recently, Kedes and Ganem (10) reported on the anti-HHV-8 activity of ACV, GCV, foscarnet, and cidofovir. Antiviral activities similar to those described in the present study for these four compounds were reported.

We next studied the intracellular phosphorylation of ACV or BVDU over a 24-h period in BCBL-1 cells that had been either pretreated for 24 h with 30 ng of TPA per ml or left untreated and that received either 2 μCi of [8-^{3}H]ACV/ml or 0.3 μCi of [2-^{3}H]BVDU/ml. High-performance liquid chromatography analysis did not reveal major differences in the phosphorylation of ACV in either the TPA-induced or uninduced cells (i.e., 1.4 and 2.0 pmol/10^6 cells of ACV monophosphate and 4.0 and 4.4 pmol/10^6 cells of ACV triphosphate in TPA-induced and uninduced cells, respectively). In contrast, ACV was efficiently phosphorylated to its mono-, di-, and triphosphate forms in HSV-1-infected Vero cells but not in mock-infected Vero cells (data not shown). TPA also did not induce major differences in the formation of BVDU mono-, di-, and triphosphate in BCBL-1 cells (i.e., 42, 8.6, and 15.4 pmol/10^6 cells and 28, 5.6, and 6.6 pmol/10^6 cells for TPA-induced cultures and uninduced cultures, respectively).

Cidofovir, a broad-spectrum anti-DNA virus agent that has recently been approved in the United States and Europe for the treatment of cytomegalovirus retinitis in AIDS patients (13), is an acyclic nucleoside phosphonate analog that does not depend on virus-encoded kinases to become antivirally active. In its diphosphorylated form, the compound selectively blocks the viral DNA polymerization process. The anti-HHV-8 activity of cidofovir reported here must depend on the selective inhibitory activity of diphosphorylated HPMPC against the HHV-8 DNA polymerase. HPMPA proved eightfold-more potent than cidofovir but has not been developed clinically. A unique characteristic of cidofovir is its long-lasting antiviral activity (14). In the clinical setting this feature translates into infrequent dosing (19). We also demonstrate here that cidofovir, but not GCV, retains marked anti-HHV-8 activity when

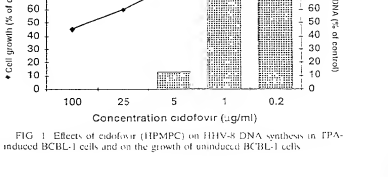

FIG. 1. Effects of cidofovir (HPMPC) on HHV-8 DNA synthesis in TPA-induced BCBL-1 cells and on the growth of uninduced BCBL-1 cells.

added to cell cultures for only a short period of time. High intracellular concentrations of the drug metabolites, even long after their levels in plasma drop below the EC_{50} for virus replication, are responsible for this effect (13). Whereas cidofovir is solely active against DNA viruses, adefovir is also a potent inhibitor of retrovirus and hepadnavirus replication (13). The oral prodrug form of PMEA, bis(pivaloylmethyl) ester (adefovir dipivoxil), is currently undergoing phase II/III trials for anti-HIV and anti-hepatitis B virus activity. Like foscarnet, adefovir inhibits both HIV and HHV-8. However, adefovir inhibits the replication of HHV-8 at a threefold-lower concentration than does foscarnet and causes 90% inhibition of HHV-8 DNA synthesis at a concentration of 90 μM, whereas foscarnet does so only at ≥449 μM. With respect to their antiherpesvirus activities, adefovir and foscarnet show a cross-resistance pattern (20). This suggests that both compounds have similarities in their mechanisms of action. This may be particularly interesting in view of the reported beneficial effect of foscarnet on KS (9, 11). The concentrations of adefovir that are reached in plasma are in the range of the EC_{50} for inhibition of KS-associated herpesvirus replication (4). Anti-HIV therapy with adefovir could thus possibly serve as an anti-HHV-8 prophylaxis.

Neither ACV, PCV, BVDU, nor H2G, molecules that depend for their activation on a viral TK, have pronounced anti-HHV-8 activity. GCV proved more effective, with a three- to eightfold-lower EC_{50}. We were not able, by using the system employed, to detect specific phosphorylation of ACV or BVDU in the TPA-induced BCBL-1 cells. This, together with the weak activity of the compounds against HHV-8, may suggest that these molecules are weak substrates for the HHV-8-encoded TK. Alternatively, or in addition, the triphosphates may be weak inhibitors of the viral DNA polymerase.

S2242 is a novel nucleoside analog that potently inhibits the replication of all herpesviruses tested, including TK-deficient strains (15). The very potent anti-HHV-8 activity of S2242 (0.1 μM) must be related to (i) the fact that the compound is efficiently phosphorylated in lymphoid cells (17) and (ii) possible potent inhibition of HHV-8 DNA polymerase by the S2242 triphosphate metabolite. Although the compound has a rather pronounced cytostatic effect on lymphoid cells, it was very well tolerated and proved highly effective in the treatment of a variety of herpesvirus infections in several animal models (16).

In conclusion, three molecules that are currently in clinical use (cidofovir, ganciclovir, and foscarnet) or under clinical study (adefovir) have marked anti-HHV-8 activity. Moreover, cidofovir has long-lasting activity against HHV-8. Finally, a molecule like adefovir that is used as an anti-HIV agent and that, in addition, has good anti-HHV-8 activity, may be particularly interesting for prophylactic use in the HIV-infected population that is at risk for acquiring HHV-8 infection.

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